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Evaluation of cellulose-based biospecific adsorbents as a stationary phase for lectin affinity chromatography

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Abstract

Macroporous cellulose Granocel was evaluated as a matrix for the immobilization of two lectins Concanavalin A (ConA) (108 kDa) and Wheat Germ Agglutinin (WGA) (36 kDa). Two different methods were employed for the immobilization of the lectins via their protein moieties by a Schiff's bases reaction. One of them results in covalent coupling of the lectin directly to the support and the other gives the attachment through a long spacer arm which benefits the immobilization of voluminous ConA molecules. The adsorbents were characterized by the glycoproteins sorption recording adsorption kinetic data and isotherms. The adsorbents demonstrated high affinity to glycoproteins with a sorption capacity in the column up to 7.4 mg/ml support and a high recovery (up to 93%). The adsorption isotherms of glucose oxidase (GOD) onto ConA adsorbents reveals an adsorption behavior with high and low affinity binding sites. The dissociation constant K_d of the ligand–sorbate complex is approximately 1×10^{-6} and 0.4×10^{-5} M, respectively. It was supposed that the second step is related to the sorption of solvated GOD onto already adsorbed GOD forming sorbate dimers.

Keywords: Lectin affinity chromatography; Biospecific adsorbents; Concanavalin A; Glucose oxidase; Adsorption isotherms

1. Introduction

Nowadays glycomics raise new challenges for affinity chromatography related with an abundance of glycoconjugates in living organisms and with scaling-up of the preparative processes. Economy, efficiency and practicality are some of the factors dictating the search of novel chromatographic supports that could contribute to enhancing the productivity of the affinity separation process. Chromatographic performance of adsorbent is the main prerequisite for an efficient and economic bioseparation of glycoconjugates.

The factors that may have impact on the chromatographic performance of affinity adsorbent are the following:

- characteristics of the matrix, such as chemistry of the matrix material, its biocompatibility, pore size and its distribution as well as the accessibility of pores for the solute [1];
- characteristics of the ligand, such as the type of the active groups, which take part in the immobilization process, the density of the ligand on the surface and their accessibility into the pores;
- ligand coupling chemistry to preserve bioactivity of the ligand immobilized as well as the type and length of spacer arm if integrated.

The prediction of the chromatographic performance is an actual topic, especially for scaling-up of the preparative and industrial processes. Theoretical approach to the prediction of the performance of affinity separations was described in detail and has been proved with experimental observations [2–6]. It revealed that basic investigations on adsorption isotherms and breakthrough curves deliver the required data. Thus, in the case of Langmuir model, the evaluated parameters such as the maximum adsorption capacity of the adsorbent (q_m) and the dissociation constants (K_d) allow the modelling of the separation process. The prediction of the behavior of affinity systems during the washing and desorption stage requires the knowledge of the

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effective dissociation constant (K_d) which reflects the strength of the ligand–sorbate binding. Thus, the kinetic data is the basis for the evaluation of a new affinity medium [7] as well as for the modelling and optimization of the sorptive bioseparation processes [8,9].

Previously [10,11] was shown that stationary phases on the cellulose-based matrix Granocel are attractive for preparative applications. In this work, the macroporous cellulose Granocel was evaluated as a matrix for lectin affinity chromatography adsorbents. The immobilization of two largely applied lectins Concanavalin A (ConA) and Wheat Germ Agglutinin (WGA) was investigated. Normally, lectins are coupled by means of cyanogen bromide method using highly toxic chemicals and dangerous procedures [2,3,12]. Recently, the application of a less toxic reaction route employing carbonyldiimidazole and *N*-hydroxysuccinimide are recommended for lectin coupling through its protein moieties [13].

In this work, two activation methods based on obtaining Schiff's bases differing in the resulting length of spacer arms were employed and compared for the coupling of the lectins. Chromatographic performances of the adsorbents were studied applying two different glycoproteins (GOD and fetuin) carrying specific terminal glyco-moieties of mannose (GOD), and *N*-acetylglucosamine (fetuin) for specific interaction with ConA and WGA, respectively.

2. Materials and methods

2.1. Materials

The lectins ConA (Type V) and WGA, the glycoproteins fetuin and glucose oxidase (GOD), sodium borohydride, sodium periodate, Bradford reagent, methyl-α-D-mannopyranoside and *N*-acetyl-D-glucosamine were purchased from Sigma (Munich, Germany). Pentaethylenehexamine (PEHA) and glutaraldehyde were purchased from Fluka (Steinheim, Germany), and 1-chloro-2,3-epoxypropane was delivered from Aldrich (Poznan, Poland). The adsorbent Agarose-ConA (carrying 8 mg protein per ml of packed gel) was obtained from Sigma (Munich, Germany). All other chemicals and solvents were of analytical reagent grade.

2.2. Methods

2.2.1. Preparation of cellulose-based matrix Granocel-4000

Cellulose-based matrix Granocel-4000 was prepared by saponification of diacetylcellulose as previously described [14]. The gel of regenerated cellulose was cut mechanically and fractionated by sieving using the fraction of the particle size of 200–315 μm for further modifications. The specific volume of the matrix is 1.6 ml/g.

2.2.2. Cross-linking of the cellulose-based matrix

Cross-linking of the matrix Granocel-4000 was performed with 1-chloro-2,3-epoxypropane (EClH) using 0.07 ml EClH per 1 g of cellulose. The sucked matrix (cellulose concentration 9.5 ± 0.5%) was suspended in the mixture of EClH and 1 M sodium hydroxide. The suspension was stirred at 50 °C for 2 h. The resulting product was washed with 2% HCl and finally with water.

2.2.3. Evaluation of exclusion limits of the matrix

Exclusion limits of the matrix were evaluated by inverse gel-permeation chromatography using the Waters HPLC system, Model R401 with a refractive index detector. 0.1% standard dextran solutions in water were injected into a 270 mm × 4.6 mm column packed with the matrix. The flow-rate was 0.3 ml/min. The dextran distribution constant K_{AV} was calculated as follows:

$$K_{AV} = \frac{V_e - V_0}{V_t - V_0} \quad (1)$$

where V_0 is the void volume of the column, V_t is the full volume, V_e is the elution volume of dextran.

Exclusion limits were determined from the calibration curve K_{AV} versus log molecular weight.

2.2.4. Activation of the matrix by the periodate method (preparation of OXY-Cel)

Two grams of sucked cellulose-based matrix Granocel-4000 was mixed with 5 ml of sodium periodate solution and kept for 2 h at room temperature stirring magnetically in the dark. After that cellulose was washed with water and with 0.1 M phosphate buffer containing 0.1 M NaCl, pH 7.4. The content of aldehyde groups was determined via the oxidation with Fehling solution.

2.2.5. Preparation of PEHA-Cel supports

2.2.5.1. Coupling of pentaethylenehexamine (PEHA) spacer arm [15]. Fifty grams of sucked cellulose Granocel-4000 was suspended in the solution containing 20 ml (46 ml) of 2% solution of NaOH and 0.8 ml (2.5 ml) PEHA. After heating up, the reaction mixture up to 50 °C, 1.1 ml (3.1 ml) of EClH and a small quantity of sodium borohydride were added. Afterwards, the reaction mixture was stirred for 3.5 h at 50 °C. The washing of the product with 0.5 M NaOH and water under slight vacuum finished the spacer integration step. The total nitrogen content was determined using the Kjeldal method.

The primary amino groups were determined by desamination of amino groups with sodium nitrite. Aminated cellulose was suspended into the solution of 0.1 M $NaNO_2$ and 0.2 M of acetic acid and heated at 70 °C for 7 h. Afterwards, the cellulose was thoroughly washed with water. The nitrogen content of primary amino groups was calculated as a difference of nitrogen content in the cellulose before and after desamination.

2.2.5.2. Reaction of aminated cellulose with glutaraldehyde. Five grams of the sucked aminated cellulose was immersed into the 9.4 ml 0.05 M phosphate buffer (pH 8.5) and 2.7 ml 25% glutaraldehyde was added. The pH value of the mixture was adjusted to 9.3 with NaOH. The suspension was kept at room temperature for 2.5 h. Afterwards, the activated cellulose was sucked and washed with 0.05 M phosphate buffer (pH 7.5).

2.2.6. Immobilization of the lectins and its kinetic

One gram of sucked activated support was immersed into 3 ml solution of either lectin ConA or lectin WGA (10 mg/ml) in buffer and kept for 15–17 h at 4 °C. Phosphate buffer (0.1 M), pH 7.4, with 0.1 M NaCl and 0.05 M phosphate buffer containing 5×10^{-3} M of Mg^{2+} (pH 7.5) were used for the immobilization of lectins onto OXY-Cel and PEHA-Cel, respectively. Afterwards, the sorbent was washed with the coupling buffer containing 0.5 M NaCl to eliminate protein–protein interactions.

The washing supernatants were collected and the protein concentration was measured by Bradford method [16]. The coupling yield was calculated from the decrease of the lectin content in the supernatant comparing to the original content applied to the matrices.

For the kinetic measurements, lectin immobilization was performed in a syringe equipped with a filter at ambient temperature. Fifteen milligrams of lectin was offered to 500 mg of sucked support in 10 ml of the coupling buffer. In defined time intervals, droplets of the supernatant were pressed out and the protein concentration was determined.

2.2.7. Blocking of unreacted aldehyde groups

$NaBH_4$ was used for the reduction of the residual aldehyde groups. One gram of the support was immersed into 2 ml of 0.1 M phosphate buffer (pH 7.4), adding 2 mg $NaBH_4$. The suspension was kept in refrigerator for 1 h. Afterwards, the adsorbent was washed with the same buffer.

2.2.8. GOD adsorption isotherms

The adsorption isotherms of the GOD onto ConA adsorbents were measured in batch systems, basically as described by Chase [4]. Three hundred milligrams of sucked adsorbents were equilibrated with known amounts of GOD with rising concentrations in 4 ml of 0.1 M acetate buffer containing 0.5 M NaCl, pH 6.0. The flasks were shaken for 3 h at 25 °C for equilibration. Then, GOD concentration in the supernatant (c^*) was determined spectrophotometrically applying the Bradford's protein assay and detecting the absorption at a wavelength of $\lambda = 595$ nm.

The amount of GOD bound to the adsorbent (q^*) was calculated as initial glycoprotein concentration minus the amount still in the soluble phase at equilibrium. The binding data were fitted to a Langmuir-type isotherm in which adsorbent load with GOD at equilibrium q^* varies with concentration of GOD at the equilibrium c^* as follows:

$$q^* = \frac{q_m c^*}{c^* + K_d} \quad (2)$$

The values of model parameters are the dissociation constant K_d and maximum adsorbent load q_m were determined from the linearized plots of Eq. (2) in which q^*/c^* are put on the ordinate and q^* at the abscissa. The intercept of plots on the abscissa reflects $-q_m$ and the slope of the line is $-1/K_d$.

2.2.9. Determination of binding capacity by dynamic procedures

The low-pressure liquid chromatography system (Bio-Rad, Munich, Germany) consisting of gradient pump, an UV detector ($\lambda = 280$ nm) and recorder were employed for affinity separations. The prepared adsorbents were packed into Bio-Rad glass columns (50 mm × 7 mm). The adsorption was performed in 0.1 M phosphate buffer, pH 7.4 adding fetuin for WGA adsorbents, and 0.1 M acetate buffer, pH 6.0 including GOD for ConA adsorbents.

After the adsorption, the removal of unbound glycoproteins was realized by washing with protein free buffer. The specific desorption was achieved by adding 0.3 M *N*-acethylglucosamine into the buffer for WGA adsorbents and 0.1 M methyl-D-mannopyranoside for ConA adsorbents. All the separation steps were carried out at ambient temperature at a flow-rate of 0.5 ml/min.

The sorption capacity of fetuin and GOD on the prepared sorbents was evaluated by applying 1 ml of the glycoprotein solution (10 mg/ml) onto the column filled with 0.8 ml of sorbent. The residence time of the fluid contacting the stationary phase was 20 min. The adsorbents were extensively washed until no more proteins were detected in the effluents. Further, the desorption processes were carried out and the eluted amount of proteins was quantified by a modified Bradford protein assay, adapted to measurements in the micromolar range. The detection was performed using the microplate reader Spectra Classic (Tecan, Crailsheim, Germany) with lectin standards.

3. Results and discussion

3.1. Matrix

The matrix plays an important role in the preparation of affinity sorbents. The ideal matrix should be macroporous, hydrophilic and neutral, stable under storage and operational conditions and should contain functional groups to allow attachment of the affinity ligands.

Cellulose shares a number of these advantages. It is cheap, inert, has a low non-specific affinity for most proteins. The hydroxyl groups on the sugar residues can be easily derivatized for the covalent attachment of a ligand. Cellulose-based matrices are of different porous structure and physical properties [17,18]. In this work, macroporous cellulose Granocel-4000 was evaluated as a matrix for lectin affinity adsorbents. Previously, stationary phases on Granocel matrix were successfully used for preparative processes in hydrophobic interaction [11] and immobilised metal affinity chromatography [10] modes. As it was determined by means of inverse gel-permeation chromatography, the pores of the matrix Granocel-4000 are accessible to molecules of molecular mass up to 2×10^6 (see Fig. 1). Considering requirements to preparative processes, the particle size of the matrix was selected of 200–315 μm.

3.2. Activation of the matrix

Two different methods were used for the activation of cellulose-based matrix Granocel-4000. Both of them result in aldehyde end-groups; however, of different location. Using the first one known as the periodate method, the aldehyde groups are obtained directly in glucose residue due to the selective oxida-

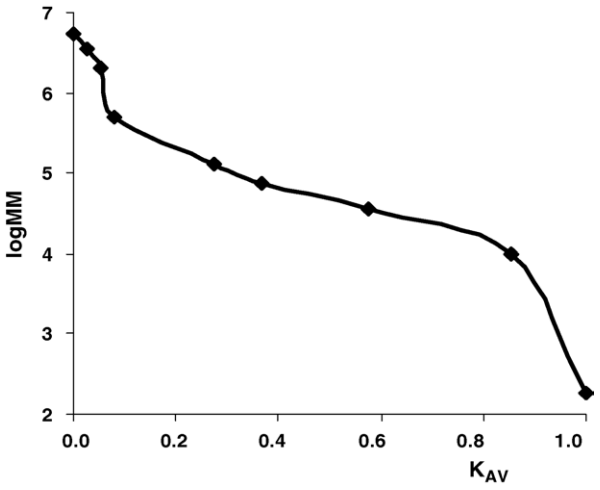


Fig. 1. Evaluation of the effective pore size of Granocel-4000 by inverse gel-permeation chromatography. K_{AV}: dextran distribution constant; log MM: log of dextran molecular weight.

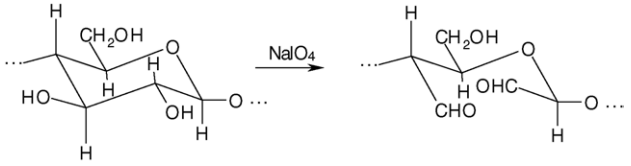


Fig. 2. Oxidation of cellulose with sodium periodate.

Table 1
Activation of Granocel matrix with sodium periodate

Concentration of $NaIO_4$ (M)	Concentration of aldehyde groups (meq/g)			
		1 h	2 h	3 h
0.1	1.12	1.12	1.24	1.55
0.2	1.43	1.86	1.92	3.35
0.3	1.92	2.23	2.23	3.84

tion of hydroxyl groups at C2 and C3 according to the reaction in Fig. 2.

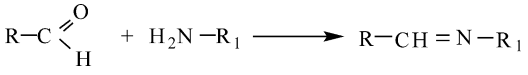
Table 1 illustrates how a high concentration of active groups is achieved even under mild conditions in 0.1 M $NaIO_4$ solution during a reaction time of 0.5 h. Having such high concentration of the active groups, a strong multipoint coupling of the ligand may be expected. For further investigations, the oxidated matrix of 1.24 meq/g of aldehyde groups was prepared on cross-linked Granocel-4000 and named OXY-Cel.

Using the second method, the aldehyde groups are introduced via a spacer arm. The procedure comprises two steps: (i) coupling of pentaethylenehexamine (PEHA) and (ii) reaction of primary amino groups of PEHA spacer arm with glutaraldehyde. An immobilized spacer adds a 24-atom arm containing hydroxyl and amino groups (see Fig. 3) onto which a further immobilization of specific ligands may be performed. The primary and secondary amino groups of the spacer arm are of weak basicity which is not dissociated at pH above 6.5–7. Thus, the possibility of non-specific ionic interaction between amino groups and proteins is negligible under the conditions that are usually applied for the chromatographic procedures.

Supports activated by the second method were named PEHA-Cel. Two supports PEHA(0.37)-Cel and PEHA(1.1)-Cel with 0.37 and 1.1% of nitrogen, respectively, were prepared for further investigations. The content of primary amino groups was found 0.10 and 0.16 meq/g, respectively.

3.3. Immobilization of lectins

The lectins ConA (108 kDa) and WGA (36 kDa) were employed as ligands for the immobilization onto the activated support. The lectins should be attached without blocking the carbohydrate binding site. The covalent attachment of the lectin onto the aldehyde activated supports takes place at the primary amino groups of the lectins. This reaction results in obtaining the so called Schiff's base:



The amount of lectin coupled to PEHA(1.1)-Cel from solutions of rising concentration is shown in Fig. 4. Although higher ligand coupling expressed in mg for 1 g of support was obtained for WGA rather than ConA, the coupled maximum for both lectins was achieved from the same concentration 10 mg/ml. A ligand density of 30 mg WGA/g support, and about 21 mg ConA/g PEHA(1.1)-Cel, respectively, was realized.

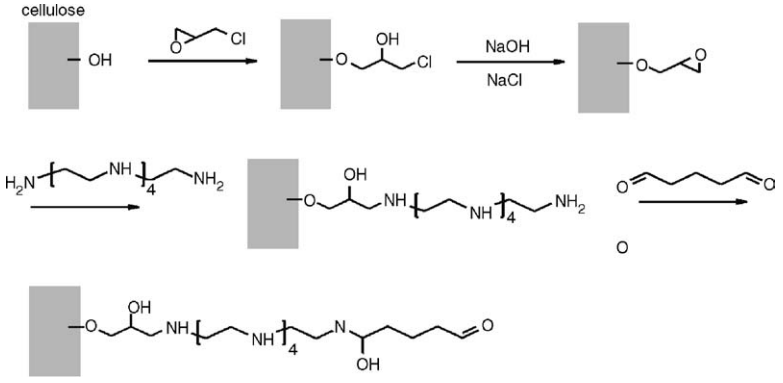


Fig. 3. Synthesis pathway of the support PEHA-Cel as described in Section 2.2.5.

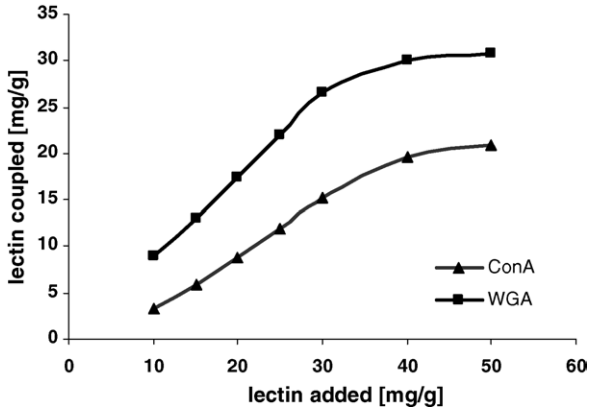


Fig. 4. Immobilization of the lectins from solutions of rising concentration on PEHA(1.1)-Cel. 0.05 M phosphate buffer containing 5×10^{-3} M of Mg^{2+} (pH 7.5) were used for the immobilization at 4 °C for 15–17 h.

To evaluate an optimal time for the completion of the ligand immobilization, the immobilization kinetic for ConA and WGA on cellulose supports was determined (Fig. 5). Results show that the reactions of both lectins onto both supports are almost completed within 4 h. Generally, the shortest coupling time is recommended for preservation of the ligand bioactivity. The prolonged coupling time is also negative because of the possible too strong multipoint attachment.

The coupling of WGA is identical on both supports and reaches up to 18 mg/ml of the support. This suggests that the spacer arm affects neither the immobilization kinetic nor the amount of coupled ligand.

ConA shows a different immobilization behavior on both supports. The faster kinetic and the higher amount of coupled ligand were achieved on the PEHA(1.1)-Cel support with the spacer arm. Several factors affect the immobilization kinetic. Mainly kinetic depends on the speed of coupling reaction and accessibility of the active groups of the support to ligand molecules. While the coupling chemistry in the case of both lectins is the same, it may be supposed that the immobilization kinetic is affected mostly by the accessibility. Aldehyde groups coupled to the long spacer arm obviously can be easier reached by the ligand molecules. Moreover, restricted accessibility of the active groups to ConA molecules may additionally result in lower ligand density in the case of OXY-Cel (9 g ConA/ml support). The benefit of a spacer arm is apparent only in the case of voluminous lectin molecules. While WGA (M = 36 kDa) is accessible to all reactive sites, steric hindrances emerge for ConA (M = 108 kDa).

Table 2
Coupling yield of the lectins on the supports

Support	Amount of coupled lectin (mg/ml)		Coupling yield (%)	
	WGA	ConA	WGA	ConA
OXY-Cel	17.5	9.0	93.3	48.0
PEHA(0.37)-Cel	9.0	7.5	48.0	40.0
PEHA(1.1)-Cel	18.5	15	98.7	80.0

The coupling yield was calculated from several points of the kinetic plateau and presented in Table 2. Although the yield achieved for WGA was only slightly higher than for ConA, it may be concluded that in all cases, both lectins were coupled with a high yield.

Differences between the results obtained on different PEHA-Cel allow to conclude that ligand density on the supports may be adjusted by: (i) altering the amount of lectin concentration used for the coupling, or (ii) by changing the content of active groups of the support.

3.4. Evaluation of the adsorbents (adsorption isotherms)

The adsorbents were characterized by equilibrium investigations of the sorption of glucose oxidase (GOD, MM 160 kDa). Adsorption isotherms can be used to describe the interactions that occur in affinity chromatography.

The results for equilibrium isotherms on ConA adsorbents are presented in Fig. 6.

Fig. 6 shows that GOD adsorption isotherms are of two steps. Favorable adsorption isotherms were found for both systems up to q^* approximately 8 mg/ml. In this range, the equilibrium values of q^* and c^* were found to fit Langmuir isotherm described

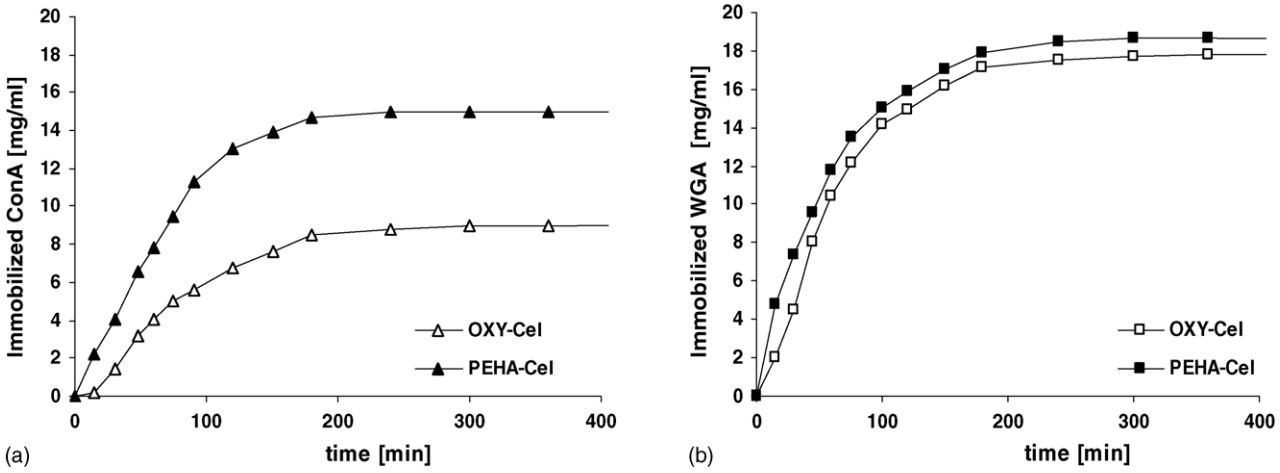


Fig. 5. Immobilization kinetics of ConA (a) and WGA (b) on OXY-Cel and PEHA(1.1)-Cel supports.

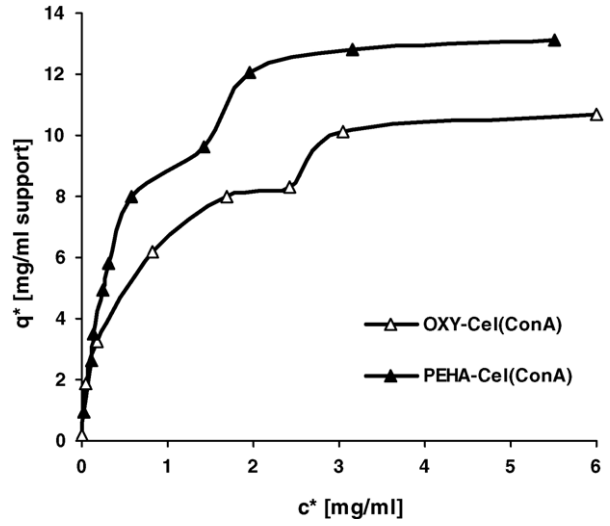


Fig. 6. Equilibrium isotherms for the adsorption of GOD onto OXY-Cel(ConA) and PEHA(1.1)-Cel(ConA). An amount of 300 mg of sucked adsorbents were equilibrated with known amounts of GOD with rising concentrations in 4 ml of 0.1 M acetate buffer containing 0.5 M NaCl, pH 6.0. The flasks were shaken for 3 h at 25 °C for equilibration.

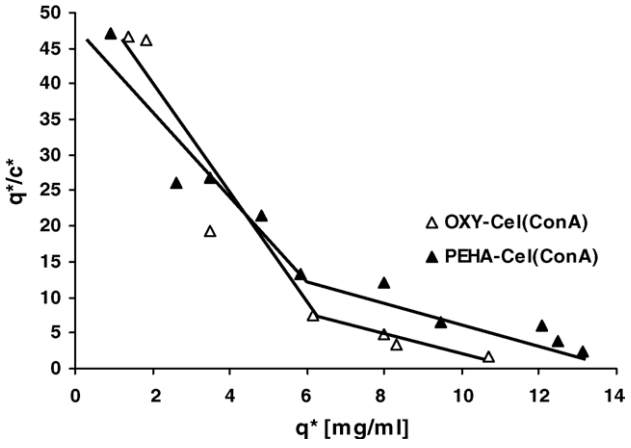


Fig. 7. Scatchard plot analysis derived from adsorption data of the ConA adsorbents. The intercept of plots on the abscissa reflects q_m and the slope of the line is $1/K_d$.

by Eq. (2). The values of the effective dissociation constants K_d and the maximum capacity of the adsorbent q_m for the affinity systems were determined as described in Section 2 from the Scatchard plots (Fig. 7) and are presented in Table 3. Both adsorbents appeared to have similar maximum capacity for binding GOD when expressed in mg of glycoprotein per ml of adsorbent, although ligand density was significantly higher in the case of PEHA(1.1)-Cel-ConA.

The comparison of molar values of K_d for the binding of GOD to the PEHA(1.1)-Cel(ConA) and OXY-Cel(ConA) suggested that GOD had similar affinity for both adsorbents. K_d reflects the strength of the GOD binding to the ligand. The dissociation constant for the affinity pair should be in the range of 10^{-4} to 10^{-8} [2]. A K_d of less than 10^{-8} requires very harsh conditions during the elution stage. With $K_d > 10^{-4}$, weak affinity chromatography characterized by fast isocratic separation is possible. Therefore, the studied affinity adsorbents show an optimum binding strength of GOD.

The second step of the adsorption isotherms is very often explained as the low affinity binding related to the weak protein–protein interaction. In our case, the sorption buffer contained 0.5 M NaCl to avoid protein interaction with adsorbed protein molecules. The dissociation constant of the second step is just five-fold higher than of the first step; consequently, it is not protein–protein interaction. The next step of the isotherms may be related to the adsorption of GOD dimers from the highly concentrated (above 10 mg/ml) glycoprotein solutions.

The results determined from the adsorption isotherms are summarized in Table 3. They indicate a good affinity between immobilized ConA and GOD for both supports.

3.5. Chromatographic performance of the affinity adsorbents

The chromatographic efficiency of prepared adsorbents was evaluated by column procedures using fetuin (MM 48,400 Da) and GOD (MM 160,000 Da) as the glycoproteins for sorption on WGA and ConA adsorbents, respectively. Determining the sorption capacity, the excess of the glycoprotein was applied to each adsorbent in the column. Having thoroughly washed the column, the glycoprotein was desorbed with the competitive sugar and quantified. The recovery was calculated from the difference between loaded, washed and eluted amounts of the glycoprotein. The results obtained are presented in Table 4. The results show that not all theoretical binding sites are accessible to the glycoprotein molecules. The sorption capacity increases with ligand density for WGA adsorbents. In the case of ConA adsorbents, high ligand density even diminishes the sorption capacity, most probably due to the steric interferences between macromolecular ligand and sorbate molecules.

The best recovery (93%) was reached on PEHA(0.37)-Cel(ConA) with low ligand density (7.5 mg/ml). Compared to commercial Agarose-ConA, PEHA(0.37)-Cel(ConA) displayed a much higher affinity to GOD.

The effect of the spacer arm on chromatographic behavior of the adsorbents in the column is also observed. OXY-Cel, where

Table 3
Characteristics of the adsorbents from the adsorption isotherms

Adsorbent	Ligand density (mg/ml)	q_m (theoretically calculated) (mg/ml)	q_m (of first step) (mg/ml)	q_m (total) (mg/ml)	K_d (of first step) (mol/l)	K_d (of second step) (mol/l)
OXY-Cel(ConA)	9.0	14.1	7.3	11.3	0.8×10^{-6}	0.4×10^{-5}
PEHA(1.1)-Cel(ConA)	15.0	23.5	8.0	14.0	1.0×10^{-6}	0.4×10^{-5}

Table 4
Chromatographic performance of the adsorbents in column

Adsorbent	Ligand density (mg/ml)	Sorption capacity		Recovery (%)
		mg/ml	% from max[a]	
PEHA(0.37)-Cel(WGA)	9.0	0.9	7.4	75
PEHA(1.1)-Cel(WGA)	18.5	2.6	10.5	66
OXY-Cel(WGA)	17.5	1.3	8.7	70
PEHA(0.37)-Cel(ConA)	7.5	7.4	62.9	93
PEHA(1.1)-Cel(ConA)	15.0	4.8	20.4	78
OXY-Cel(ConA)	9.0	3.1	22.0	70
Agarose-ConA	8.0	4.4	35.3	65

[a] Theoretical maximum capacity was calculated from ligand density considering one binding sites per ligand molecule.

the lectin is coupled directly to cellulose chain, exhibited lower sorption capacity comparing to the adsorbents of the similar ligand density with the spacer arm. This effect is evident for both ConA and WGA adsorbents.

4. Conclusions

Cellulose-based matrix Granocel-4000 proved to be a promising support for the preparation of biospecific adsorbents for lectin affinity chromatography. Adsorbents obtained by the immobilization of Concanavalin A (ConA) and Wheat Germ Agglutinin (WGA) on the Granocel matrix have showed affinity to glycoproteins with high capacity and recovery. Chromatographic characteristics of biospecific adsorbent, such as sorption capacity, recovery, significantly depend on accessibility of the sorbate to ligand sites. Adsorptive performance can be improved by proper choice of the ligand density on the surface of adsorbent. Considering the accessibility, low ligand density is preferred in the case of large lectin and higher ligand density is recommended if the size of lectin molecule is rather small.

The applicability of the ligand coupling methods based on obtaining the Shiff's bases for the immobilization of the lectins on polysaccharide supports was demonstrated.

It was shown that spacer arm affected ligand coupling kinetic as well as the chromatographic behavior of the adsorbents obtained.

Scatchard plot analysis of the adsorption isotherms suggested the presence of two different interactions between immobilized ConA and glucose oxidase (GOD). The values of the dissociation constants of both bindings allow suggesting that this behavior may be a consequence of dimerization of GOD in highly concentrated solutions.

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